

What Is Claimed Is:

1. A method for functionally classifying a protein, said method comprising:

5 (a) screening one or more of a multiplicity of different molecules for their ability to modify the stability of said protein, wherein modification of the stability of the protein indicates that the molecule binds to the protein;

10 (b) generating an activity spectrum for said protein from said screening of step (a), wherein said activity spectrum reflects a subset of molecules, from said multiplicity of different molecules, that modify the stability of said protein and therefore are ligands that bind to the protein;

(c) comparing said activity spectrum for said protein to one or more functional reference spectrum lists; and

(d) classifying said protein according to the set of molecules in said multiplicity of different molecules that modify the stability of said protein.

15 2. The method of claim 1, wherein said screening step (a) comprises:

(a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) treating said protein in each of said multiplicity of containers to cause said protein to unfold;

20 (a3) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(a4) generating an unfolding curve for said target molecule for each of said containers; and

25 (a5) comparing each of said unfolding curves in step (d) to (1) each of said other unfolding curves and to (2) the unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and

(a6) determining whether any of said multiplicity of different molecules modifies the stability of said protein, wherein a modification in stability is indicated by a change in said unfolding curve.

3. A method for functionally classifying a protein, said method comprising:

(a) screening one or more of a multiplicity of different molecules known to bind to a particular class of proteins for their ability to modify the stability of said protein, wherein modification of the stability of the protein indicates that the molecule binds to the protein;

(b) generating an activity spectrum for said protein from said screening of step (a), wherein said activity spectrum reflects a subset of molecules, from said multiplicity of different molecules, that modify the stability of said protein and therefore are ligands that bind to the protein; and

(c) classifying said protein as a member of said class of proteins if said one or more of said multiplicity of different molecules modify the stability of said protein.

4. The method of claim 3, wherein said screening step (a) comprises:

(a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) treating said protein in each of said multiplicity of containers to cause said protein to unfold;

(a3) measuring in each of said containers a physical change associated with the unfolding of said target molecule;

(a4) generating an unfolding curve for said target molecule for each of said containers; and

(a5) comparing each of said unfolding curves in step (d) to (1) each of said other unfolding curves and to (2) the unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and

(a6) determining whether any of said multiplicity of different molecules modifies the stability of said protein, wherein a modification in stability is indicated by a change in said unfolding curve.

5 5. A method for functionally classifying a protein, said method comprising:

classifying said protein according to the set of molecules in a multiplicity of different molecules that modify the stability of said protein.

10 6. A method for functionally classifying a protein that is capable of unfolding due to a thermal change, said method comprising:

(a) screening one or more of a multiplicity of different molecules for their ability to shift the thermal unfolding curve of said protein, wherein a shift in the thermal unfolding curve of the protein indicates that the molecule binds to the protein;

15 (b) generating an activity spectrum for said protein from said screening of step (a), wherein said activity spectrum reflects a subset of molecules, from said multiplicity of different molecules, that shift the thermal unfolding curve of said protein and therefore are ligands that bind to the protein;

(c) comparing said activity spectrum for said protein to one or more functional reference spectrum lists; and

20 (d) classifying said protein according to the set of molecules in said multiplicity of different molecules that shift the thermal unfolding curve of said protein.

7. The method of claim 6, wherein said screening step (a) comprises:

25 (a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) heating said multiplicity of containers from step (a1);

(a3) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;

(a4) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers; and

5 (a5) comparing each of said unfolding curves in step (a4) to (1) each of said other thermal unfolding curves and to (2) the thermal unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and

10 (a6) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said protein.

8. The method of claim 7, wherein said comparing step (a5) comprises ranking said molecules in said multiplicity of different molecules for said protein according to the ability of each of said multiplicity of different molecules to shift the thermal unfolding curve of said protein.

15 9. The method of claim 7, wherein in said heating step (a2), said multiplicity of containers is heated simultaneously.

20 10. The method of claim 7, wherein said step (a4) further comprises determining a midpoint temperature (T_m) from the thermal unfolding curve; and wherein said step (a5) further comprises comparing the T_m of each of said unfolding curves in step (a4) to (1) the T_m of each of said other thermal unfolding curves and to (2) the T_m of the thermal unfolding curve obtained for said target protein in the absence of any of said different molecules.

11. The method of claim 7, wherein said step (a3) comprises measuring the absorbance of light by said contents of each of said containers.

12. The method of claim 7, wherein said step (a1) comprises contacting said protein with a fluorescence probe molecule present in each of said multiplicity of containers and wherein said step (a3) comprises

5 (i) exciting said fluorescence probe molecule, in each of said multiplicity of containers, with light; and

(ii) measuring the fluorescence from each of said multiplicity of containers.

10 13. The method of claim 12, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers one container at a time.

14. The method of claim 12, wherein said step (a3)(ii) further comprises measuring the fluorescence from a subset of said multiplicity of containers simultaneously.

15 15. The method of claim 12, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers simultaneously.

16. The method of claim 7, wherein said step (a3) comprises

20 (i) exciting tryptophan residues in said protein, in each of said multiplicity of containers, with light; and

(ii) measuring the fluorescence from each of said multiplicity of containers.

17. The method of claim 7, wherein said multiplicity of containers in step (a1) comprises a multiplicity of wells in a microplate.

18. A method for functionally classifying a protein that is capable of unfolding due to a thermal change, said method comprising:

5 (a) screening one or more of a multiplicity of different molecules known to bind to a particular class of proteins for their ability to shift the thermal unfolding curve of said protein, wherein a shift in the thermal unfolding curve of the protein indicates that the molecule binds to the protein;

10 (b) generating an activity spectrum for said protein from said screening of step (a), wherein said activity spectrum reflects a subset of molecules, from said multiplicity of different molecules, that shift the thermal unfolding curve of said protein and therefore are ligands that bind to the protein; and

(c) classifying said protein as a member of said class of proteins if said one or more of said multiplicity of different molecules shift the thermal unfolding curve of said protein.

15 19. The method of claim 18, wherein said screening step (a) comprises:

(a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) heating said multiplicity of containers from step (a1);

(a3) measuring in each of said containers a physical change associated with the thermal unfolding of said target molecule resulting from said heating;

20 (a4) generating a thermal unfolding curve for said target molecule as a function of temperature for each of said containers; and

(a5) comparing each of said unfolding curves in step (a4) to (1) each of said other thermal unfolding curves and to (2) the thermal unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and

25 (a6) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said protein.

20. The method of claim 19, wherein said comparing step (a5) comprises ranking said molecules in said multiplicity of different molecules for said protein according to the ability of each of said multiplicity of different molecules to shift the thermal unfolding curve of said protein.

5 21. The method of claim 19, wherein in said heating step (a2), said multiplicity of containers is heated simultaneously.

22. The method of claim 19, wherein said step (a4) further comprises determining a midpoint temperature (T_m) from the thermal unfolding curve; and wherein said step (a5) further comprises comparing the T_m of each of said unfolding curves in step (a4) to (1) the T_m of each of said other thermal unfolding curves and to (2) the T_m of the thermal unfolding curve obtained for said target protein in the absence of any of said different molecules.

23. The method of claim 19, wherein said step (a3) comprises measuring the absorbance of light by said contents of each of said containers.

15 24. The method of claim 19, wherein said step (a1) comprises contacting said protein with a fluorescence probe molecule present in each of said multiplicity of containers and wherein said step (a3) comprises

(i) exciting said fluorescence probe molecule, in each of said multiplicity of containers, with light; and

20 (ii) measuring the fluorescence from each of said multiplicity of containers.

25. The method of claim 24, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers one container at a time.

26. The method of claim 24, wherein said step (a3)(ii) further comprises measuring the fluorescence from a subset of said multiplicity of containers simultaneously.

27. The method of claim 24, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers simultaneously.

28. The method of claim 19, wherein said step (a3) comprises

(i) exciting tryptophan residues in said protein, in each of said multiplicity of containers, with light; and

(ii) measuring the fluorescence from each of said multiplicity of containers.

29. The method of claim 18, wherein said multiplicity of containers in step (a1) comprises a multiplicity of wells in a microplate.

30. A method for functionally classifying a protein capable of unfolding due to a thermal change, said method comprising:

classifying said protein according to the set of molecules in a multiplicity of different molecules that shift the thermal unfolding curve of said protein.